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COMMENTS:

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SONGWON

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Via Federal Express

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Subject: Notice in Accordance with Section 8 (e): Results of an algal growth inhibition test with Octyl-3,5-di-tert-butyl-4-hydroxy-hydrocinnamate (CAS No. 125643-61-0)

Dear Sir/Madam: **CONTAINS NO CBI**

This letter is to inform you of the results of an algal growth inhibition test with Octyl-3,5-di-tert-butyl-4-hydroxy-hydrocinnamate (CAS No. 125643-61-0) in the green alga Desmodesmus subspicatus (D. subspicatus).

The substance was subject to an algal growth inhibition test in the green alga *D. subspicatus*. The certificated GLP-study was conducted according to OECD Guideline 201, is well documented and therefore judged to be of high quality and reliability (Klimisch 1).

Substance preparation:
Pre-study solubility work conducted indicated that the test material was practically insoluble in water using traditional methods of preparation. Based on this information the test material was categorised as being a 'difficult substance' as defined by the OECD Guidance Document on Aquatic Toxicity Testing of Difficult Substances and Mixtures (OECD 2000).
A media preparation trial was conducted using both solvent spike and saturated solution methods with prolonged stirring. Overall, the saturated solution method resulted in the highest test material concentration of 0.52 mg/L, indicating this to be the limit of water solubility of this material under test conditions. Therefore, a modification of the standard method for the preparation of aqueous media was performed (exposure of organisms to a saturated solution of the test material). The test samples have been shown to be stable in the test medium.

Algae cultures
Liquid cultures of the freshwater unicellular alga *D. subspicatus* CCAP 276/20 were maintained by the periodic replenishment of culture medium (under constant agitation and

illumination at 24 ± 1°C). Prior to the start of the test sufficient master culture was added to culture media to give an initial cell density of approximately 10^3 cells/mL. The flasks were kept under constant agitation and constant illumination at 24 ± 1°C until the algal cell density was approximately 10^4– 10^5 cells/mL. Toxicity is determined by comparison of viability in the presence of test material treated cultures relative to the negative control.

Range-finding test:
The test concentrations to be used in the initial definitive test were determined by a preliminary range-finding test, exposing *D. subspicatus* cells to a series of nominal test concentrations of 0.0052, 0.052 and 0.52 mg/L for a period of 72 hours. No effect on biomass integral or yield at all test concentrations employed, but inhibition of growth rate was observed to have occurred at 0.52 mg/L.

Initial test:
Based on this information an initial test was conducted at test concentrations of 0.0325, 0.065, 0.13, 0.26 and 0.52 mg/L. The biological data obtained from this initial test indicated that no effect on growth rate, yield or biomass integral occurred at all test concentrations employed, unlike in the range-finding test where inhibition of growth rate was observed.

Definitive Test:
It was, therefore, considered appropriate to conduct the definitive test as a limit test. *D. subspicatus* was exposed to the test material (1 litre of the saturated solution inoculated with 14 mL algal suspension, initial nominal cell density of 4 x 10^3 cells/mL, no significant dilution effect on the final test concentration) at a mean measured test concentration of 0.29 mg/L (six replicate flasks) for 72 hours, under constant illumination and shaking at a temperature of 24 ± 1°C. The saturated test material solution was prepared by stirring an excess (50 mg/L) of test material in culture medium at a temperature of 21°C for 24 hours. After the stirring period any undissolved test material was removed by centrifugation to produce a saturated solution of the test material with a nominal concentration of 0.29 mg/L.
Samples were taken at 0, 24, 48 and 72 hours and the cell densities determined for each control and treatment group.
Analysis of the test preparations revealed a measured concentration of approximately 0.52 mg/L during the medial preparation trial and a mean measured concentration of 0.29 mg/L at 0 hours in the definitive test.
Given that the preliminary stability analyses showed that the test material was stable the decline in measured test concentration was considered to be due to adsorption to algal cells.

Results of positive control substance:
Exposure of *D. subspicatus* to the reference material potassium dichromate (samples taken at 0, 28, 52 and 72 hours), gave an ErC50 (0-72 h) of 0.49 mg/L, an EyC50 (0-72 h) of 0.22 mg/L and an EbC50 (0-72 h) of 0.23 mg/L. The Lowest Observed Effect Concentration based on inhibition of growth rate, yield and biomass integral were 0.25, 0.125 and 0.125 mg/L, respectively and the No Observed Effect Concentrations were 0.125, 0.0625 and 0.0625 mg/L, respectively. The results from the positive control with potassium dichromate were within the normal range for this reference material.

Validity criteria:
Concerning the validity criteria of the test, the cell concentration, the mean coefficient of variation for section by section specific growth rate and for average specific growth rate (0 - 72 h) for the control cultures satisfied the validation criteria given in the OECD Guideline.

The pH deviation in the control cultures was also within the limits given in the Test Guidelines.

Results:
The growth rate (r), yield (y) and biomass (b) of *D. subspicatus* were not affected by the presence of the test material. Accordingly for the inhibition of growth rate, the inhibition of yield, the inhibition of biomass integral, the ErC10, ErC 20 and ErC 50 were above 0.29 mg/L. There were no statistically significant differences (P>0.05), between the control and 0.29 mg/L test group and, therefore, the "No Observed Effect Concentration" (NOEC) based on growth rate was 0.29 mg/L.

The EC50 values based on the geometric mean measured test concentrations were greater than 0.18 mg/L and correspondingly, the No Observed Effect Concentration for growth rate, yield and biomass integral was 0.18 mg/L.

Conclusion: The test material was considered to be hazardous the aquatic environment. Care should be taken in the interpretation of the results of the study based on geometric mean measured test concentrations (giving a "worst case" analysis of the data) as, whilst there was a significant decline in measured test concentrations over the test period, the test material was shown to be stable in aqueous medium and hence the decline was considered to be due to absorption to algal cells. As such it may be considered that the algal cells were exposed to nominal concentrations of test material throughout the test period.
However, the test material was considered to be Category Acute 1 according to Globally Harmonized System of Classification and Labelling of Chemicals (GHS), fourth revised edition, United Nations, 2011. It should be labelled with the signal word **Warning**, **the Hazard Statement: very toxic to aquatic life** and the **Symbol**:

Songwon International understands that reporting of the results from this study under TSCA 8(e) is in accordance with EPA's policy.

If you have any questions, please call James McGinley at 281-648-1585

Sincerely,

James J. McGinley
President
Songwon International – Americas, Inc.

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